

Anti-inflammatory properties of the PI3K pathway are mediated by IL-10/DUSP regulation

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ABSTRACT

Resolution of inflammation is an important hallmark in the course of infectious diseases. Dysregulated inflammatory responses may have detrimental consequences for the affected organism. Therefore, tight regulation of inflammation is indispensable. Among numerous modulatory signaling pathways, the PI3K/PTEN signaling pathway has been proposed recently to be involved in the regulation of innate immune reactions. Here, we attempted to elucidate molecular mechanisms that contribute to the modulatory properties of the PI3K signaling pathway in inflammation. PTEN-deficient macrophages, which harbor constitutively active PI3Ks, were analyzed in response to gram-negative bacteria and PAMPs such as LPS. PTEN-deficient cells showed reduced inflammatory cytokine production, which was accompanied by reduced MAPK signaling activation in early- as well as late-phase activation. Simultaneously, we found increased levels of the MKP DUSP1, as well as the anti-inflammatory cytokine IL-10. Our data suggest that differential DUSP1 regulation coupled with enhanced IL-10 production contributes to the anti-inflammatory properties of the PI3K pathway. *J. Leukoc. Biol.* **88**: 1259–1269; 2010.

Introduction

Tight regulation during onset as well as resolution of the innate immune response to pathogens or PAMPs is of utmost importance for the affected host. In clinical settings, secondary bacterial infections with subsequent complications in patients are a major cause for morbidity and mortality. Gram-negative bacteria such as *Acinetobacter baumannii* or *Pseudomonas aeruginosa* account for severe nosocomial infec-

tions, especially in immune-compromised patients admitted to intensive care units. On the other hand, overwhelming inflammatory responses to bacterial pathogens, which are often seen in cases of severe sepsis or acute lung injury, may lead to increased tissue damage, organ failure, and ultimately, death [1].

Several signaling molecules are implicated in down-modulation of inflammatory pathways, such as IRAK-M, suppressors of cytokine signaling family members, and the IL-10/Jak/Stat3 pathway [2–4]. More recently, the PI3K signaling pathway, which is activated by TLR agonists, and downstream signaling molecules have been described by us and others [5–10] to exert pronounced, anti-inflammatory effects by way of effective limitation of proinflammatory gene expression.

The PI3K signaling pathway is activated within minutes upon stimulation of macrophages by TLR agonists. The active enzyme, which consists of a catalytic p110 and a regulatory p85 subunit, generates phosphatidylinositol-(3,4,5)-trisphosphate, which leads to the recruitment of downstream kinases such as AKT. PI3K/AKT signaling serves pleiotropic functions, such as cell motility, phagocytosis, and cell survival [11]. Recent advances in the understanding of the role of PI3K in innate immune regulation using genetic models suggest potent anti-inflammatory properties. Enhanced and sustained PI3K activity effectively suppresses MAPK signaling [5, 6]. The molecular mechanism of the mode of inhibition of inflammatory pathways of PI3K is still incompletely understood.

As a model to mimic constitutively active PI3K activity specifically in macrophages, we make use of a conditional deletion of the endogenous PI3K antagonist and tumor suppressor PTEN. Ablation of the *pten* gene by a cre-lox system driven by the LysM promoter leads to enhanced and sustained PI3K signaling activity in monocytes, macrophages, and neutrophil granulocytes [12].

Here, we show that activation of PTEN-deficient macrophages by heat-killed bacteria leads to suppressed MAPK activity in early- as well

Abbreviations: BALF=BAL fluid, cre=cyclization recombination, DUSP=dual-specificity phosphatase, EGR-1=early growth response protein 1, IRAK-M=IL-1R-associated kinase M, KO=knockout, LysM=lysozyme M, MKP=MAPK phosphatase, p=phosphatase, Pam₃CSK₄=palmitoyl-3-cysteine-serine-lysine-4, poly I:C=polyinosinic:polycytidylic acid, PTEN=phosphatase and tensin homologue deleted on chromosome 10, TIR=TLR/IL-1R

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as in late-phase activation. Transcription and release of proinflammatory cytokines such as TNF- α and IL-6 are reduced. In contrast, anti-inflammatory IL-10 is up-regulated significantly in resting as well as stimulated PTEN-deficient macrophages. Furthermore, we found that among several investigated anti-inflammatory candidate genes, such as IRAK-M, a MKP is differentially regulated upon TLR stimulation. This group of phosphatases, denominated as DUSPs, dephosphorylate p-Thr as well as p-Ser/p-Tyr sites on MAPKs [13]. In fact, DUSP1/MKP-1 has been shown previously to exert potent, innate immune-modulatory properties [14, 15].

We propose that increased PI3K activity in PTEN-deficient macrophages results in pronounced, anti-inflammatory, cellular properties through differential DUSP1 and IL-10 regulation.

MATERIALS AND METHODS

Mice

Floxed PTEN mice were kindly provided by Tak W. Mak (University of Toronto, Toronto, Canada) [16], LysM cre recombinase transgenic mice were a kind gift from Randall Johnson (UCSD, La Jolla, CA, USA) [17]. Inter-crossed mice were backcrossed to a C57BL/6J background for at least eight generations. Mice deficient for p85 α were provided by Shigeo Koyasu (Keio University School of Medicine, Tokyo, Japan). This strain is backcrossed to a C57BL/6J background for at least 13 generations. Littermate-controlled experiments were performed using 8- to 12-week-old male mice. All animal studies were approved and comply with institutional guidelines (BMWF-66.009/0103-C/GT/2007).

Genotyping

For identification, mice were ear-marked. Murine ear tissue, which we extracted, was lysed in PCR-lysis buffer, and direct PCR was performed using GoTaq DNA polymerase (Promega, Madison, WI, USA); PTEN primer: forward, 5'-ctcctactcattctccc-3', reverse, 5'-actcccacatgacaaac-3'; cre primer: forward, 5'-tcgcgattatctctatctctagc-3', reverse, 5'-gctcgaccagtttagttacc-3'.

Harvest of primary macrophages

Thioglycollate-elicited peritoneal macrophages were isolated from PTEN^{MC-KO} and PTEN^{MC-WT} controls. In brief, 2 ml 4% thioglycollate medium was injected i.p., and macrophages were harvested 3 days later by peritoneal lavage with 5 ml Ringer's solution. Macrophages were counted and seeded at a concentration of 10⁶ cells/ml in RPMI-1640 medium (Invitrogen Corp., Carlsbad, CA, USA), supplemented with 10% FCS, 1% penicillin/streptomycin/fungizone, and 1% L-glutamine. After 2 h, medium was exchanged to remove non-adherent cells, and adherent cells were allowed to recover overnight.

Stimulation of primary macrophages

Thioglycollate-elicited peritoneal macrophages were stimulated in vitro with 100 ng/ml ultra-pure LPS, FSL-1, Pam₃CSK₄, and poly I:C (Invivogen, San Diego, CA, USA) or 10⁷ CFU/ml heat-killed *A. baumannii* for the indicated time-points.

A. baumannii (ATCC 17961, American Type Culture Collection, Manassas, VA, USA) were grown to midlogarithmic phase at 37°C using LB broth (Difco, Detroit, MI, USA), harvested by centrifugation at 1500 g for 15 min, and washed twice in sterile isotonic saline. Then, bacteria were killed at 60°C for 1 h. Finally, heat-killed bacteria were resuspended in sterile isotonic saline at a concentration of 10⁹ CFU/ml and stored at -80°C.

To determine precise CFUs, bacterial counts were determined by plating serial tenfold dilutions on sheep-blood agar plates.

Murine rIL-10 was obtained from ImmunoTools (Friesoythe, Germany).

Western blotting and ELISA

Macrophage cell lysates were separated by SDS-PAGE, blotted to Immobilon PVDF transfer membrane (Millipore, Bedford, MA, USA), and

probed with primary antibodies against PTEN, I κ B, p-I κ B, p65, p-p65, p-ERK, p38, p-p38, p-JNK, and p-DUSP1 (all Cell Signaling Technology, Beverly, MA, USA); DUSP1 (M-19 clone, Santa Cruz Biotechnology, Santa Cruz, CA, USA); and β -actin (Sigma Chemical Co., St. Louis, MO, USA). For detection, a secondary antibody conjugated with HRP (Amersham, Piscataway, NJ, USA) was used. Membranes were developed using the chemiluminescence reagent assay SuperSignal West Femto and exposed in the FluorChem HD2 chemiluminescence imager (Alpha Innotech Corp., San Leandro, CA, USA). Bands were analyzed according to their molecular weight. Actin, non-p-p65, and non-p-p38 were used for normalization.

For ELISA measurements BAL and lung tissue samples were analyzed for TNF- α , IL-6, and IL-10 using the DuoSet ELISA kits (R&D Systems, Minneapolis, MN, USA).

Quantitative real-time RT-PCR

Total RNA was isolated from naïve and stimulated macrophages using TRIzol reagent (Invitrogen Corp.), according to the manufacturer's protocol, and reverse-transcribed. Semiquantitative real-time PCR was performed using Fast SYBR Green Mastermix (Applied Biosystems, Foster City, CA, USA) and the StepOne Real-Time PCR system (Applied Biosystems). Transcription levels of target genes were assayed in duplicates, normalized to GAPDH levels, and depicted as fold induction of unstimulated macrophages. The following primer pairs (Table 1), which we have used, have been designed by us, with the exception of DUSP primer sequences [18]. TLR2 and TLR4 primers have been provided by Dr. Harald Esterbauer (Medical University of Vienna, Austria).

Acinetobacter pneumonia and BAL

A nonlethal *Acinetobacter pneumonia* was performed in mice as described previously [19, 20]. Briefly, *A. baumannii* was obtained from the American Type Culture Collection (ATCC 17961) and grown to log-phase. Mice were short-term-anesthetized with isoflurane (Abbott, Wiesbaden, Germany), and 50 μ l bacterial suspension (\sim 10⁷ CFUs) was inoculated intranasally. Mice were killed 6 h postinfection, and bilateral BAL was performed by instilling 1 ml sterile saline as reported previously [21]. Therefore, the trachea was exposed through a midline incision and cannulated with a sterile 22-gauge Abbocath-T catheter (Abott). BALFs were subsequently spun down at 1250 rpm for 5 min, the supernatants frozen at -70°C for cytokine analysis, and the cell pellet lysed in sample buffer for Western blot analysis. Whole lungs were harvested and homogenized at 4°C in 4 vol sterile saline using a tissue homogenizer (Biospec Products, Bartlesville, OK, USA). CFUs were determined from serial dilutions of lung homogenates plated on blood agar plates and incubated at 37°C for 16 h before colonies were counted. Cytokines were quantified in lung homogenates and BALFs using ELISA DuoSets (R&D Systems) as described earlier [19].

Statistical analysis

Data were analyzed by GraphPad Prism 4 software using unpaired Student's *t* test followed by post hoc tests when appropriate. Values are expressed as mean \pm sd. Criteria for significance for all experiments were *P* < 0.05.

RESULTS

Stimulation of PTEN-deficient macrophages with heat-killed *A. baumannii* leads to reduced expression of proinflammatory cytokines

Expression and subsequent release of cytokines and chemokines are indispensable during the course of infectious diseases. Recognition of pathogens by immune-competent cells will lead to the immediate release of cytokines to amplify the initial response to the potential threat.

TABLE 1. Q-PCR Primers

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
A20	GACCATGGCACAACCTCATCTCA	GTTAGCTTCATCCAACCTTTGCGGCATTG
DUSP1	GGATATGAAGCGTTTTTCGGCT	GGATTCTGCACTGTCAGGCA
DUSP2	TGGAGATCCTGTCTCTCCTG	CCTTCCGAGAAGCGTGATAG
DUSP4	CTACCTCGGCAGTGCCTATC	GACGGGGATGCACTTGTACT
DUSP5	TGGATGTGAAGCCCACCTCA	CGCACTTGGATGCGTGGTAG
DUSP10	CGCCTACTTGATGAAGCACA	AGGTTGCGGGAAATAATTGG
GAPDH	GGTCGTATTGGGCGCTGGTCACC	CACACCCATGACGAACATGGGGGC
IL-6	TGCAAGTGCATCATCGTTGTTC	CCACGGCCTTCCCTACTTCA
IL-10	TGGCCCAGAAATCAAGGAGC	CAGCAGACTCAATACACACT
IRAK-M	TTTGAATGCAGCCAGTCTGA	GCATTGCTTATGGAGCCAAT
PTEN	ACACCGCCAAATTTAACTGC	TACACCAGTCCGTCCCTTTC
TLR2	TCAGCTCACCGATGAAGAAGC	TGTAACGCAACAGCTTCAGGAG
TLR4	TCTGGCATCATCTTCATTGTCC	GCGATAACAATCCACCTGCTG
TNF- α	GAACTGGCAGAAGAGGCACT	GGTCTGGCCCATAGAACTGA

First, we measured the deletion efficiency in PTEN-deficient macrophages at the level of mRNA transcription. We can show that thioglycollate-elicited peritoneal macrophages derived

from floxed PTEN LysM cre-positive mice express PTEN mRNA only marginally, as measured by quantitative real-time RT-PCR (Fig. 1A). Furthermore, we show by Western blotting that

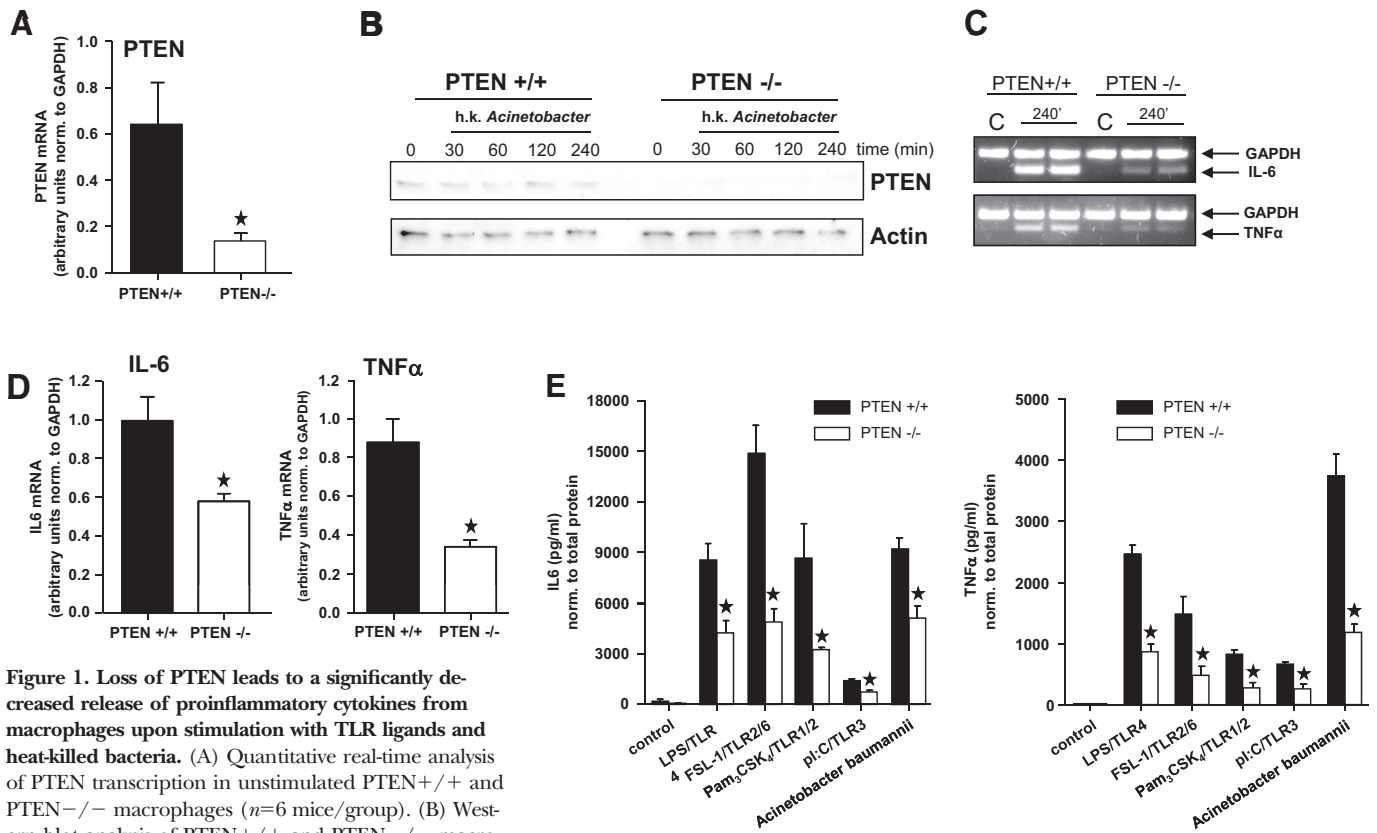


Figure 1. Loss of PTEN leads to a significantly decreased release of proinflammatory cytokines from macrophages upon stimulation with TLR ligands and heat-killed bacteria. (A) Quantitative real-time analysis of PTEN transcription in unstimulated PTEN^{+/+} and PTEN^{-/-} macrophages ($n=6$ mice/group). (B) Western blot analysis of PTEN^{+/+} and PTEN^{-/-} macrophages stimulated with heat-killed (h.k.) *A. baumannii* for the indicated time-points. Blots were probed with antibodies specific for PTEN. (C) IL-6 and TNF- α mRNA levels from PTEN^{+/+} and PTEN^{-/-} macrophages 240 min upon stimulation with heat-killed *A. baumannii* are shown by semiquantitative PCR analysis. GAPDH levels serve as a loading control ($n=2$ mice/group). C, Control. (D) Quantitative real-time analysis of IL-6 and TNF- α transcription in PTEN^{+/+} and PTEN^{-/-} macrophages 240 min upon stimulation with heat-killed *A. baumannii* ($n=3$ mice/group). Target genes were normalized to GAPDH; results are not efficiency-corrected. Data are presented as mean and sd. (E) ELISA analysis of IL-6 and TNF- α release into supernatants in triplicates of PTEN^{+/+} and PTEN^{-/-} macrophages overnight upon stimulation with LPS, FSL-1, Pam₃CSK₄, poly I:C (pI:C), and heat-killed *A. baumannii* ($n=3$ mice/group). Statistical significance is indicated by * $P < 0.05$.

PTEN protein expression in macrophages is down-regulated to undetectable levels, although PTEN protein levels are not changed in WT macrophages stimulated with heat-killed bacteria (Fig. 1B). Previously, we could demonstrate that PTEN deficiency in macrophages leads to increased and sustained PI3K activity [6, 7].

To analyze the effect of PTEN deficiency during the inflammatory response to clinically relevant pathogens in macrophages, we used heat-killed, gram-negative *A. baumannii* in vitro. We measured macrophage mRNA transcription of TNF- α and IL-6 by semiquantitative PCR and quantitative real-time RT-PCR. Bacteria-induced mRNA expression of both cytokines was reduced significantly in PTEN-deficient macrophages (Fig. 1C and D) as compared with littermate control WT cells. Data were confirmed on the protein level by ELISA analysis of supernatants (Fig. 1E). To determine whether the observed effect of PTEN deficiency on the expression of proinflammatory cytokines is specific for a receptor, we performed experiments using various TLR ligands such as LPS/TLR4, FSL-1/TLR2/6, Pam₃CSK₄/TLR1/2, and poly I:C/TLR3. At least the TLR agonists that we used led to a significantly reduced cytokine expression in PTEN-deficient macrophages (Fig. 1E).

Down-regulation of proinflammatory cytokines by PTEN deficiency is independent of changes in TLR expression and NF- κ B activation or expression of other anti-inflammatory molecules

Initially, it was unclear whether differential TLR expression accounted for the modulated pathogen responsiveness. Therefore,

we measured the steady-state levels of the most important TLRs for the recognition of *Acinetobacter* in this respect. Gram-negative bacteria will be recognized preferentially by TLR4. Additionally, TLR2 may contribute in pathogen detection. Expression of both receptors was unaltered in unstimulated macrophages. Modulation of the PI3K/PTEN pathway does not influence mRNA levels of TLR2 and TLR4 (Fig. 2A).

Previously, we have shown that PTEN is involved in the regulation of MAPK activation upon LPS/TLR4 stimulation [6]. To analyze early (30 min and earlier) as well as late (60 min and later) effects of constitutively active PI3K on the TLR-activated NF- κ B pathway, PTEN-deficient macrophages and WT littermate control cells were stimulated with heat-killed *A. baumannii* for the indicated time-points. We determined expression and post-translational modifications of the inhibitor of NF- κ B, I κ B, and p65, which is one the major NF- κ B transcription factor subunits. As expected, we detected phosphorylation of I κ B and p65 within 30 min upon cell stimulation. I κ B and p65 phosphorylation was only marginally different (Fig. 2B). Similarly, we did not find any overt differences in degradation as well as resynthesis of I κ B in PTEN-deficient or WT macrophages stimulated with heat-killed bacteria (data not shown). These findings indicate that the TLR/NF- κ B pathway is nearly unaffected by PTEN deficiency upon treatment of macrophages with heat-killed bacteria.

Moreover, we analyzed the expression pattern of two inhibitory NF- κ B-dependent candidate genes upon stimulation with heat-killed bacteria and LPS in PTEN $-/-$ macrophages and littermate control WT cells. We chose IRAK-M and A20, as both inter-

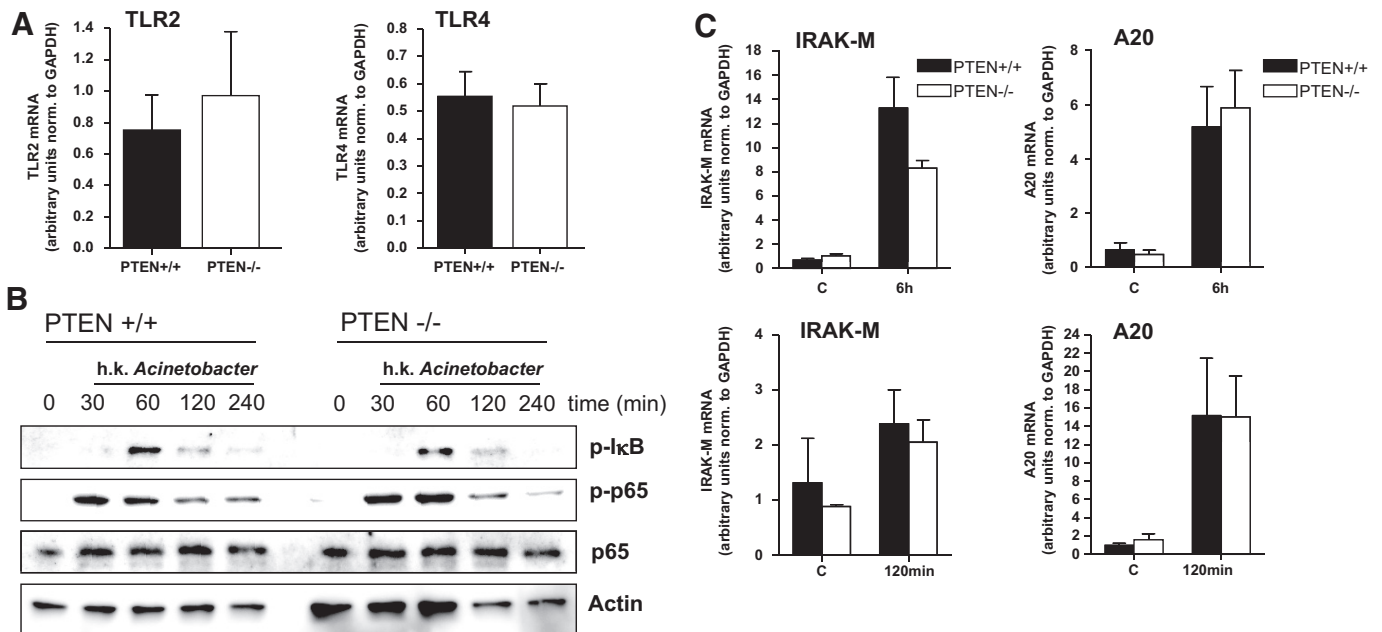


Figure 2. Differential transcription of PRRs, activation of the NF- κ B pathway or two potent anti-inflammatory mediators do not contribute to the anti-inflammatory effects of PTEN deficiency in macrophages. (A) Quantitative real-time analysis of TLR2 and TLR4 transcription in unstimulated PTEN^{+/+} and PTEN^{-/-} macrophages ($n=6$ mice/group). (B) Western blot analysis of PTEN^{+/+} and PTEN^{-/-} macrophages stimulated with heat-killed *A. baumannii* for the indicated time-points. Blots were probed with antibodies specific for p-I κ B, p-p65, and p65. Actin serves as a loading control. Representative Western blots of $n = 3$ mice/group are shown. (C) Quantitative real-time analysis of IRAK-M and A20 transcription in *Acinetobacter*-stimulated PTEN^{+/+} and PTEN^{-/-} macrophages ($n=6$ mice/group). Target genes were normalized to GAPDH; results are not efficiency-corrected. Data are presented as mean and SD. C, Control.

ferred with the TLR4/NF- κ B signaling pathway [3, 22]. Interestingly, mRNA levels of both genes were not changed in macrophages, with or without PTEN in response to heat-killed *Acinetobacter* 30 min and 120 min poststimulation, although mRNA transcription was induced markedly (Fig. 2C). We conclude from these data that these genes do not contribute to the anti-inflammatory properties that we observed in the cell with increased and sustained PI3K activity.

PTEN deletion in macrophages results in reduced early as well as late MAPK activity and increased MKP expression and stability in response to TLR agonists and IL-10

The TLR signaling pathway uses the MAPK signaling complex, which consists of ERK, p38, and JNK, to activate transcription factors such as Ets Like gene-1 (ELK-1)/EGR-1, activating transcription factor 2, and AP-1, respectively. Each of those factors importantly contributes to immediate inflammatory gene expression during the very early onset of the innate immune response [23–25].

We analyzed the effect of PTEN deficiency on early- and late-phase activation of the MAPKs by heat-killed *A. baumannii*. Previously, we could show that positive or negative modulation of the PI3K signaling pathway results in an immediate effect on MAPK activation [6]. In this study, we investigated effects on the regulation of late-phase MAPK activation by PTEN as well as molecules that might be involved.

Macrophages with ablated PTEN gene and WT control cells were stimulated with heat-killed *Acinetobacter*. We found that the initial as well as delayed activation of ERK1/2, p38, and JNK1/2 was limited in PTEN $^{-/-}$ macrophages. Interestingly, bacteria-induced MAPK activation is sustained only in WT cells, as PTEN $^{-/-}$ macrophages showed reduced p-MAPK levels at later time-points, up to 240 min postinduction (Fig. 3A).

To analyze the mechanisms by which PTEN deficiency alters MAPK activation, we sought to elucidate the PI3K-dependent regulation of enzymes that regulate the MAPK activity and phosphorylation. It is known that members of the MKP family or DUSPs effectively limit the activity of ERK, p38, and JNK with varying specificity. DUSPs have been described as down-modulatory molecules in the innate immune response to LPS in vitro and in vivo [15, 26, 27].

To determine the possibility that members of the DUSP family are intricately involved in the anti-inflammatory PTEN phenotype that we described above, we sought to measure expression of potential DUSP candidates. *Acinetobacter*-stimulated macrophages were tested for their ability to express DUSP1, which is one of several MKPs involved in an innate immune mechanism [28]. DUSP1 expression on mRNA and protein levels was up-regulated immediately upon activation by heat-killed bacteria. Indeed, PTEN deficiency led to a substantial increase in DUSP1 mRNA (Fig. 3B and C).

DUSP1 protein levels are very low in resting macrophages. However, we could detect slightly but still significantly elevated baseline protein levels of DUSP1 in PTEN-deficient macrophages as compared with WT cells (Fig. 3C and D, and see Fig. 6C). Heat-killed *Acinetobacter* could stimulate DUSP protein synthesis within 60 min (Fig. 3D).

Stimulating WT and PTEN-deficient macrophages with 10^7 CFU heat-killed *Acinetobacter* led to the discovery that in line with previously obtained mRNA data, DUSP1 protein is substantially up-regulated in PTEN-deficient macrophages 60 min postincubation, and at the same time, ERK phosphorylation was reduced (Fig. 3C). In addition, we found that concomitantly, DUSP1 phosphorylation was enhanced in the PTEN-deficient cells (Fig. 3C). As described by Brondello et al. [29], phosphorylation of DUSP1 leads to reduced proteasomal degradation. These findings indicate that not only expression but also protein stability might be increased in macrophages with sustained PI3K activity (Fig. 3C).

Moreover, in these experiments, we inhibited the MEK/ERK pathway by the pharmacologic inhibitor U0126 to analyze the influence of this MAPK on DUSP expression and/or DUSP post-translational modification. We found that ERK in particular but also MAPK in general are indispensable for DUSP expression in response to bacterial activation (Fig. 3C, upper panel). Similar results could be obtained by us with specific inhibitors against p38 and JNK (data not shown). Furthermore, we tried to circumvent the previously observed DUSP1 expression issue by stimulating the cells with *Acinetobacter* for 60 min and subsequently inhibit MAPKs for 15 min. We found that even during this short period of time, the inhibition of ERK led to a substantial decrease in DUSP1 protein (Fig. 3C, lower panel). At the downside, we could not further investigate post-translational modifications, as inhibition of MAPK completely abrogated DUSP1 protein expression.

In additional time-course experiments, we found that DUSP1 expression at early (15 min and 30 min; Fig. 3D, bottom) as well as late time-points (120 min; data not shown) was increased substantially in PTEN-deficient macrophages.

Diminished PI3K signaling leads to reduced DUSP1 expression

Conversely, we analyzed the effect of TLR-mediated DUSP1 expression in cells with greatly diminished PI3K activity to show that the effects that we have observed in PTEN-deficient cells are indeed a result of the modulation of PI3K activity. For that matter, we made use of p85 α -deficient macrophages, which are the regulatory subunits of active class Ia PI3Ks. We can show that heat-killed *Acinetobacter*-induced DUSP1 expression is reduced in cells with diminished PI3K activity (Fig. 4A), whereas simultaneously, p38 activation, as measured by phosphorylation, was greatly enhanced in p85 α -deficient macrophages (Fig. 4B).

DUSP1 mRNA stability is not affected by PTEN deficiency

To block transcription and analyze transcriptional stability of DUSP1 mRNA, we treated unstimulated macrophages with the transcriptional inhibitor Actinomycin D (5 μ g/ml) for the indicated time-points (Fig. 5A). Under these conditions, no difference in the message stability between PTEN $+/+$ and PTEN $^{-/-}$ could be observed by us.

Furthermore, we activated macrophages with heat-killed bacteria to initiate DUSP1 transcription for 90 min. Then, Actino-

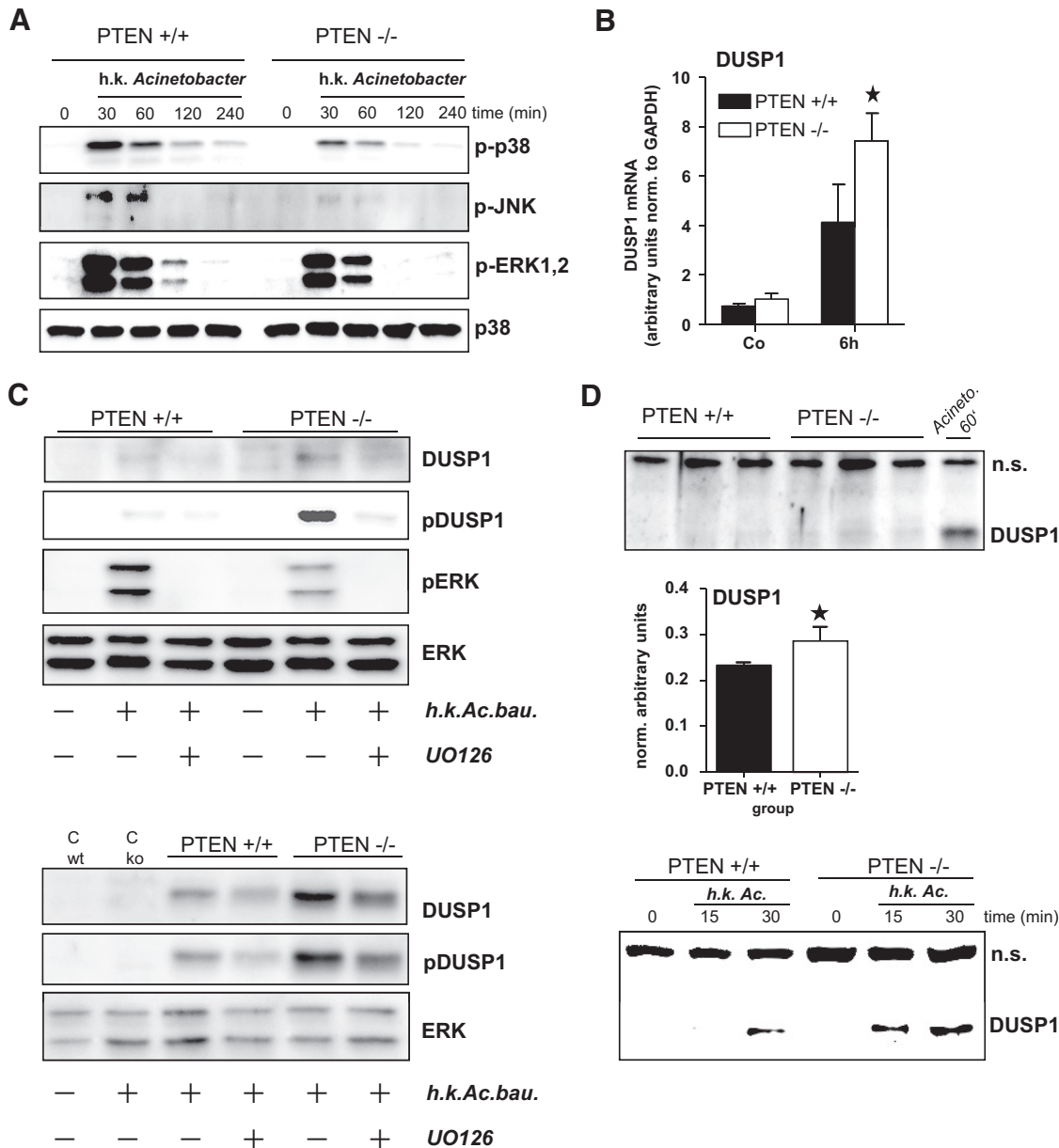


Figure 3. Early- and late-phase activation of the MAPK pathway is reduced, and transcription and post-translational modification of DUSP1 is enhanced in PTEN-deficient macrophages. (A) Western blot analysis of PTEN^{+/+} and PTEN^{-/-} macrophages stimulated with heat-killed *A. baumannii* for the indicated time-points. Blots were probed with antibodies specific for p-ERK, p-p38, and p-JNK. p38 serves as a loading control. Representative Western blots of *n* = 3 mice/group are shown. (B) Quantitative real-time analysis of DUSP1 transcription in PTEN^{+/+} and PTEN^{-/-} macrophages stimulated with heat-killed *A. baumannii* for 240 min (*n*=3 mice/group). (C) Western blot analysis of *Acinetobacter* (CFU 10⁷/ml)-stimulated (60 min) PTEN^{+/+} and PTEN^{-/-} macrophages with DUSP1, p-DUSP1, and p-ERK antibodies. Macrophages were preincubated for 15 min with the MEK1 inhibitor UO126 (10 μM; upper panel) or postincubated with UO126 after 60 min *Acinetobacter* stimulation for a further 15 min (lower panel). ERK served as loading control. C, control. (D) Western blot analysis of unstimulated PTEN^{+/+} and PTEN^{-/-} macrophages with DUSP1 antibodies. Quantification of Westerns is shown (*n*=6). Bacteria-induced DUSP1 is included as a positive control (top and middle panels). Western blot analysis of a time course (up to 30 min) of *Acinetobacter*-stimulated PTEN^{+/+} and PTEN^{-/-} macrophages with DUSP1 antibodies (bottom panel). Representative Western blots of *n* = 3 mice/group are shown; **P* < 0.05.

mycin D (5 μg/ml) was added to the cells to prevent further transcription for 30 min. We did not find any significant difference in the DUSP1 message stability between stimulated PTEN-deficient macrophages and WT littermate control cells. Similar results were obtained with prolonged Actinomycin D

inhibition for up to 4 h (data not shown). DUSP1 transcription was set to 100% in both groups (Fig. 5B). At the same time, we could detect profound differences in DUSP1 mRNA at 90 min in these stability experiments, showing significantly more DUSP1 transcripts in PTEN-deficient macrophages.

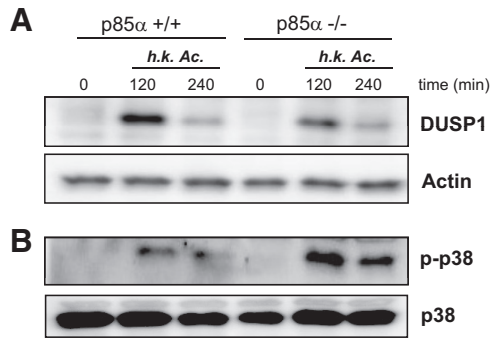


Figure 4. p85 α deficiency leads to reduced DUSP1 expression.

(A) Western blot analysis of p85 α +/+ and p85 α -/- macrophages stimulated with heat-killed *A. baumannii* for the indicated time-points. Blots were probed with antibodies specific for DUSP1. Actin served as loading control. (B) Blots were probed with antibodies specific for p-p38 and p38, and p38 served as a loading control. Representative Western blots of $n = 3$ mice/group are shown.

Moreover, we included the analysis of TNF- α transcription 90 min postbacterial stimulation. In contrast to DUSP1, PTEN-deficient cells produced significantly less TNF- α (Fig. 5C).

Increased IL-10 expression in PTEN-deficient macrophages

IL-10 is among the most prominent anti-inflammatory cytokines released during inflammatory reactions. Analysis of stimulated macrophages revealed that IL-10 is differentially regulated in PTEN-deficient cells. We found that mRNA levels as well as IL-10 cytokine levels released into the supernatant of cultured KO macrophages are increased substantially as compared with cells derived from littermate control mice (Fig. 6A and B).

To test the idea that IL-10 or other important inflammatory stimuli may contribute to DUSP1 expression, we stimulated cells with IL-10 and LPS. IL-10 has already been described to regulate DUSP expression in a positive-feedback loop [14]. Interestingly, we found that LPS as well as IL-10 stimulated DUSP1 in macrophages. This effect was markedly enhanced in PTEN-deficient cells (Fig. 6C). These data indicate that PTEN is involved in the regulation of TLR-dependent IL-10 expression. Furthermore, IL-10 may contribute to the enhanced DUSP1 expression that we observe in resting as well as stimulated PTEN-deficient macrophages.

The role of PTEN in the elimination of pathogens in a murine model of *Acinetobacter*-induced pneumonia

To investigate the effect of PTEN deficiency in vivo, we decided to apply a model of acute lung injury by intranasal infection with *A. baumannii*. Infection in healthy WT mice is nonlethal, although a pronounced, acute inflammatory response occurs. Usually, the infection is contained within 24 h, and bacterial burden is reduced significantly in immune-competent mice.

In our in vivo model, we decided to analyze two time-points. We killed infected mice at an early time-point (6 h postinfection). Here, we aimed to analyze the initial bacterial infection and increased lung inflammation by cytokine measurements.

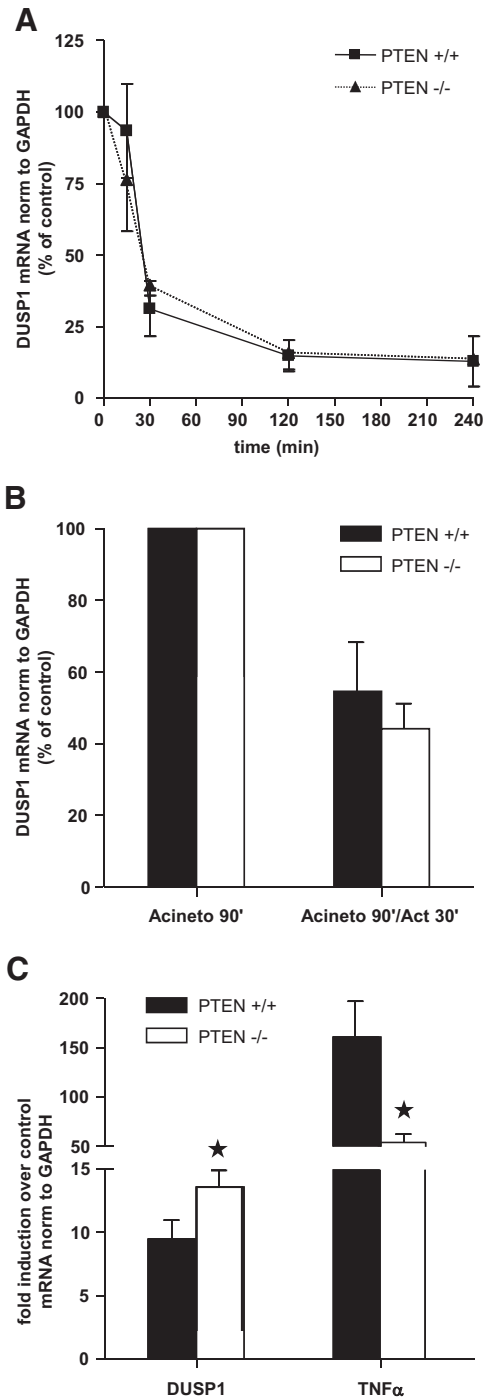


Figure 5. PTEN deficiency does not influence DUSP1 mRNA stability. (A) mRNA stability assays were performed in unstimulated PTEN+/+ and PTEN-/- macrophages using Actinomycin D (5 μ g/ml) for the indicated time-points. DUSP1 mRNA levels were measured by quantitative PCR. (B) PTEN+/+ and PTEN-/- macrophages were stimulated with heat-killed *A. baumannii* for 90 min (Acineto 90'). Transcription was inhibited with Actinomycin D for 30 min (Act 30'). DUSP1 mRNA levels were measured by quantitative PCR. (C) PTEN+/+ and PTEN-/- macrophages were stimulated with heat-killed *A. baumannii* for 90 min. DUSP1 and TNF- α mRNA were measured by quantitative PCR; * $P < 0.05$.

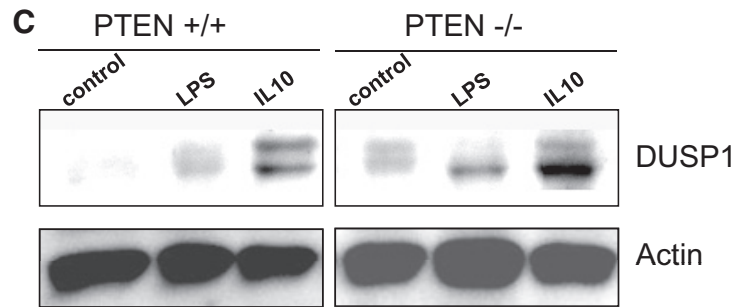
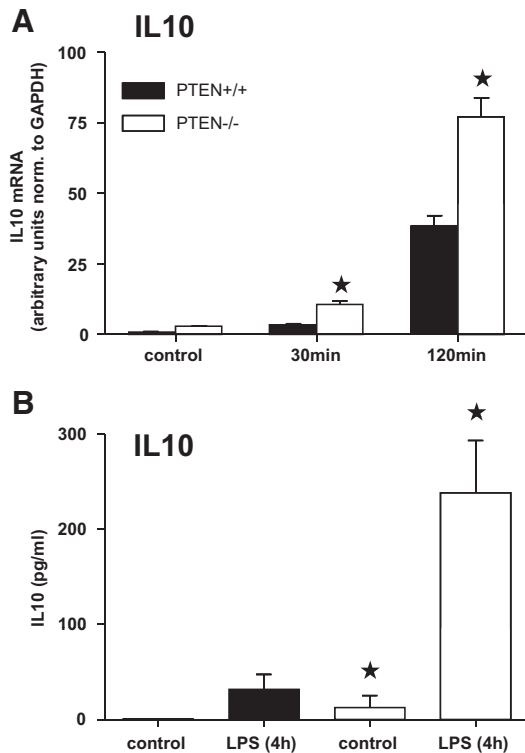


Figure 6. IL-10 is up-regulated in PTEN-deficient macrophages and induces DUSP1 expression. (A and B) Quantitative real-time analysis of IL-10 transcription and ELISA analysis of IL-10 release in PTEN^{+/+} and PTEN^{-/-} macrophages stimulated with gram-negative LPS for the indicated time-points ($n=3$ mice/group). (C) Western blot analysis of PTEN^{+/+} and PTEN^{-/-} macrophages stimulated with gram-negative LPS or IL-10 for 240 min. Actin serves as a loading control. Target genes in quantitative real-time analysis were normalized to GAPDH; results are not efficiency-corrected. Data are presented as mean and sd. Statistical significance is indicated by $*P < 0.05$.

Next, we chose a late time-point, 24 h postinfection. Here, we expected to detect containment of the infection by reduced bacterial burden in the lung.

Interestingly, the bacterial burden in the PTEN-deficient mice showed significantly enhanced pathogen persistence 24 h postinoculation (Fig. 7A). One explanation for that might be a diminished inflammatory response, which is required to limit bacterial growth.

Indeed, we found increased levels of TNF- α and IL-6 in the alveolar lavage in WT mice infected with *A. baumannii*. Myeloid PTEN deficiency resulted in reduced levels of TNF- α 6 h postinfection, similar to the in vitro data shown above (Fig. 7B). In contrast to our cell culture experiments, IL-6 was unaffected, indicating that cells other than macrophages are the main source for IL-6 in this model (Fig. 7C). Interestingly, IL-10 was undetectable in the bronchial lavage (data not shown). Only when we measured IL-10 in whole lung homogenates did we find that IL-10 levels were increased significantly in PTEN-deficient mice at all time-points measured (Fig. 7D). Moreover, 6 h postinfection, we analyzed the expression of DUSP1 in the alveolar lavage cells, which are predominately macrophages. At this time, in addition to alveolar macrophages, neutrophil granulocytes infiltrated the infected alveoli. In line with in vitro data obtained in macrophages, we found elevated levels of DUSP1 in these cells (Fig. 7E). Analysis of the bacterial CFUs at 24 h revealed that probably as a result of the diminished immune response, outgrowth of bacteria could not be prevented in PTEN-deficient mice (Fig. 7A).

This indicates that probably as a result of hampering the inflammatory response in PTEN-deficient mice by increased DUSP1 and IL-10 expression, the bacterial infection cannot be contained properly.

DISCUSSION

Despite the controversial data that have been published about the role of PI3K in innate immune regulation, we can show that constitutive activation of the PI3K signaling pathway by cell type-specific deletion of its antagonist PTEN results in reduced, proinflammatory gene expression in murine macrophages and in an infectious in vivo model of acute lung injury. It is known that the PI3K pathway serves pleiotropic rather proinflammatory functions in the immune response to invading pathogens, such as phagocytosis and chemotaxis. Nevertheless, we speculate that PI3K and its counterpart PTEN are actively involved in the modulation of the magnitude of inflammatory responses, essentially contributing to resolution of inflammation.

What molecules are responsible for the reduced, proinflammatory gene expression by hyperactivation of the PI3K signaling pathway in macrophages?

Previously, Laird et al. [30] could provide evidence that the interaction of the PI3K subunit p85(α,β) with the TLR4/TIR domain-containing adaptor protein/MyD88 negatively regulates signaling. The authors propose that sustained interaction/activation negatively regulates TLR signaling [30]. This fits to our own observations in PTEN-deficient macrophages, showing that enhanced as well as sustained PI3K activity reduces TLR4-mediated, proinflammatory gene expression [6, 31]. In addition, we could provide evidence that the anti-inflammatory properties of PI3K do not discriminate between different TLR agonists. Broad-range activation, achieved by *Acinetobacter*, as well as specific activation by TLR ligands, such as LPS or FSL-1, are inhibited significantly by PTEN-deficient macrophages (see Fig. 1E).

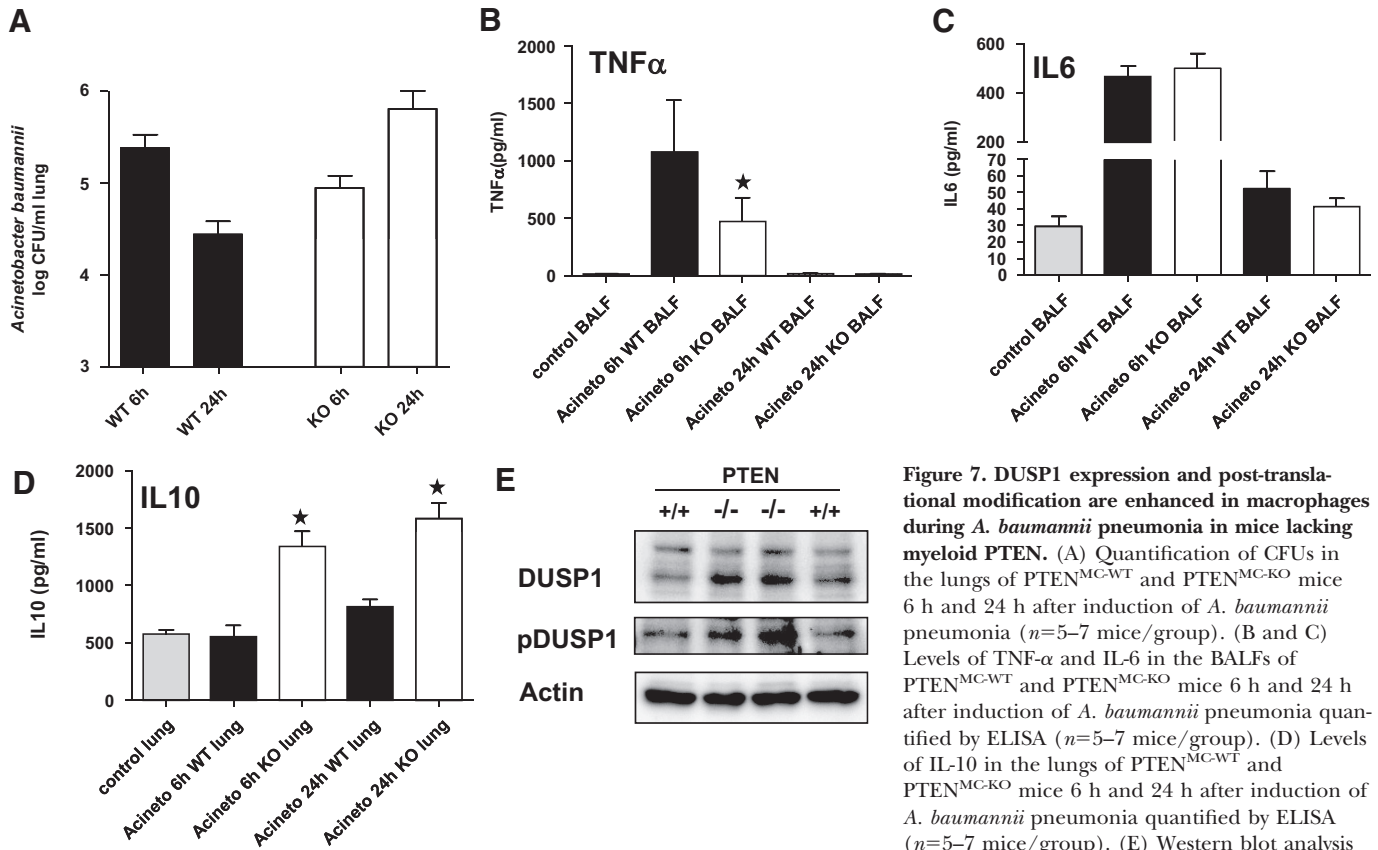


Figure 7. DUSP1 expression and post-translational modification are enhanced in macrophages during *A. baumannii* pneumonia in mice lacking myeloid PTEN. (A) Quantification of CFUs in the lungs of PTEN^{MC-WT} and PTEN^{MC-KO} mice 6 h and 24 h after induction of *A. baumannii* pneumonia ($n=5-7$ mice/group). (B and C) Levels of TNF- α and IL-6 in the BALFs of PTEN^{MC-WT} and PTEN^{MC-KO} mice 6 h and 24 h after induction of *A. baumannii* pneumonia quantified by ELISA ($n=5-7$ mice/group). (D) Levels of IL-10 in the lungs of PTEN^{MC-WT} and PTEN^{MC-KO} mice 6 h and 24 h after induction of *A. baumannii* pneumonia quantified by ELISA ($n=5-7$ mice/group). (E) Western blot analysis

of alveolar macrophages of PTEN^{MC-WT} and PTEN^{MC-KO} mice 24 h after induction of *A. baumannii* pneumonia. Blots were probed with antibodies specific for p-DUSP1 and DUSP1 ($n=2$ mice/group). Actin serves as a loading control. Representative Western blots of $n = 3$ mice/group are shown. Data are presented as mean and SD. Statistical significance is indicated by * $P < 0.05$.

Now, we are able to extend these findings to PI3K-dependent MAPK regulation by specific MKPs.

DUSP1 gene regulation

Our findings support the notion that the PI3K pathway down-regulates the TLR-induced MAPK activity by way of up-regulation of DUSPs, which are specific MKPs, whereas the TLR/NF- κ B pathway activation, as measured by I κ B degradation and p65 phosphorylation, is nearly unaffected. Our findings match with previously published data by Martin et al. [32], showing that GSK3 β dampens the innate immune response by CREB-binding protein transcription cofactor competition rather than by direct NF- κ B inhibition.

Modulated DUSP gene expression at the early phase, 15–30 min poststimulation, and late phase of activation, 60–120 min, is mediated by sustained PI3K activity. PTEN deficiency leads to enhanced and prolonged PI3K activity, which we have shown earlier [6]. Even baseline DUSP expression is increased in PTEN-deficient macrophages. Moreover, we could exclude the possibility that alterations in the post-transcriptional message stability are the cause for differential DUSP expression in PTEN-deficient cells (see Fig. 5).

In addition, we prove that the PTEN-mediated effects on DUSP1 as well as p38 are dependent on PI3K using cells

deficient for the regulatory subunit of PI3K p85 α (see Fig. 4A and B).

Several DUSP family members are implicated in immune regulatory mechanisms. In particular, DUSP1 is described as an essential factor in innate immunity modulating the activity MAPKs by dephosphorylation upon TLR stimulation [26, 27, 33]. In this respect, DUSP1 shows a substrate preference for p38 (p38>JNK>>ERK) [34]. Intriguingly, in our hands, all analyzed MAPKs, namely ERK, JNK, as well as p38, are affected by PTEN deficiency. This may indicate that more than one DUSP protein is involved in the observed phenotype. Indeed, we found DUSP2 differentially transcribed on the mRNA level (data not shown). As DUSP2 is implicated in the regulation of ERK as well as p38 [28], we believe that the differential DUSP2 expression in PTEN-deficient macrophages may account for the general inhibition of MAPK activation. However, the involvement of DUSP2 in the anti-inflammatory effects of PI3K still needs to be clarified experimentally.

DUSP1 protein modification

We found substantially increased phosphorylation of DUSP1 at Ser359 in macrophages with increased PI3K activity. The post-translational modification of DUSP1 may be mediated directly or indirectly by sustained PI3K activity. It has been shown that phosphorylation of this site together with Ser364 confers stabil-

ity of the enzyme by reduced proteasomal degradation [29]. This indicates that in addition to the observed effects on the transcriptional level, post-translational events on DUSP1 may contribute to the reduced MAPK activity in PTEN-deficient cells, although this has to be clarified in future studies (see our schematic model, Fig. 8).

IL-10 regulation by PTEN

Furthermore, we could provide evidence that the PI3K/PTEN pathway is involved in the regulation of steady-state levels as well as TLR-induced IL-10 levels in macrophages. IL-10 has been proven to directly affect the expression and synthesis of several proinflammatory markers via STAT3 activation [4, 35]. We found that noninduced as well as TLR ligand-induced, PTEN-deficient macrophages produce significantly more IL-10 than WT cells. This may indicate that IL-10 autocrine action may contribute to the observed anti-inflammatory phenotype. However, so far, we could not find a consistent effect of IL-10 blocking antibodies on the PTEN-dependent immune-modulatory functions (data not shown), which may exclude an anti-inflammatory feedback loop by IL-10 on the IL-10-producing macrophages. Further analysis of PI3K/PTEN-regulated IL-10 release and downstream signal transduction is needed to draw any final conclusions. Nevertheless, increased synthesis of IL-10 by macrophages will have an impact on macrophages and the surrounding tissue in case of an acute inflammatory event, which is likely to occur in our in vivo model of *Acinetobacter*-induced pneumonia. As IL-10 has been shown to influence DUSP1 [14], we think that IL-10 should have some effect on the regulation of the MAPKs as well (see our schematic model, Fig. 8).

Negative effect on bacterial clearance in PTEN-deficient mice

In previous studies as well as in the current work, we could show in vitro that macrophage-specific deletion of PTEN re-

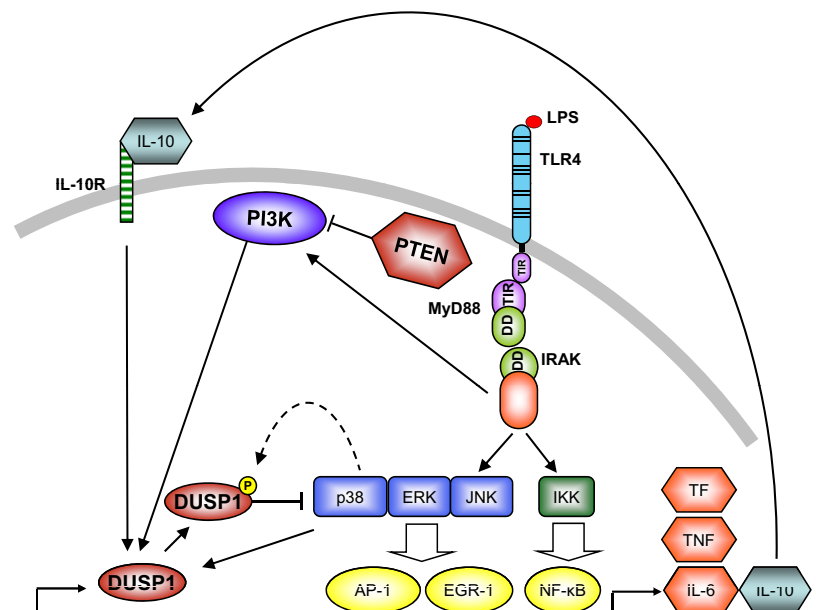
sults in reduced *Acinetobacter*- as well as LPS/TLR4-induced proinflammatory gene expression [6, 7]. In line with the observed in vitro data, analysis of LysM cre-positive floxed PTEN mice and littermate controls infected with *A. baumannii* in lungs revealed that TNF- α release into the alveolar compartment was reduced significantly by PTEN deficiency. Although the reduced inflammatory response might be beneficial for the host tissue reducing cellular damage, the bacterial burden was markedly increased. Under normal circumstances, WT mice mount an immediate immune response and hence, can successfully eradicate the *Acinetobacter* infection within 24 h [19, 20]. In contrast, PTEN-deficient mice show enhanced bacterial counts. Moreover, PTEN is described to play an indispensable role in the recruitment of neutrophils to the site of infection [36]. We can show in another model of acute lung injury that PTEN deficiency leads to reduced neutrophil alveolar influx [31]. Our findings indicate that immune suppression and possible diminished neutrophil recruitment led to dysfunctional bacterial killing.

We conclude from our data that PI3K and its antagonist PTEN are part of important regulatory signaling complexes regulating important anti-inflammatory molecules such as DUSP1 in the resolution of inflammatory responses and essentially contribute to orchestrate the innate immune defense to bacterial pathogens.

AUTHORSHIP

The authors contributed as follows: P.G.: data collection, data analysis, design, and review of the manuscript; K.B.: data collection, data analysis, and review of the manuscript; E.H.: data collection, data analysis, and review of the manuscript; U.M.: data collection, data analysis, and review of the manuscript; B.D.: data analysis; B.M.: data analysis; S.K.: design and review of the manuscript; B.R.B.: design and review of the manu-

Figure 8. Model for the anti-inflammatory properties of PI3K mediated by DUSP1 and IL-10. Activation of the MAPKs by bacterial products is limited by the PI3K signaling pathway via differential regulation of the MKP DUSP1. Activation of the PI3K pathway, which is regulated efficiently by the lipid phosphatase PTEN, leads to enhanced IL-10 synthesis. IL-10 in turn can influence DUSP1 expression, thereby limiting proinflammatory gene expression. DD, Death domain; TF, tissue factor.



script; G.S.: conception, design, data collection, data analysis, and manuscript preparation.

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REFERENCES

- Matthay, M. A., Zimmerman, G. A. (2005) Acute lung injury and the acute respiratory distress syndrome: four decades of inquiry into pathogenesis and rational management. *Am. J. Respir. Cell Mol. Biol.* **33**, 319–327.
- Dalpke, A., Heeg, K., Bartz, H., Baetz, A. (2008) Regulation of innate immunity by suppressor of cytokine signaling (SOCS) proteins. *Immunobiology* **213**, 225–235.
- Kobayashi, K., Hernandez, L. D., Galan, J. E., Janeway Jr., C. A., Medzhitov, R., Flavell, R. A. (2002) IRAK-M is a negative regulator of Toll-like receptor signaling. *Cell* **110**, 191–202.
- Moore, K. W., de Waal, M. R., Coffman, R. L., O'Garra, A. (2001) Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* **19**, 683–765.
- Günzl, P., Schabbauer, G. (2008) Recent advances in the genetic analysis of PTEN and PI3K innate immune properties. *Immunobiology* **213**, 759–765.
- Luyendyk, J. P., Schabbauer, G. A., Tencati, M., Holscher, T., Pawlinski, R., Mackman, N. (2008) Genetic analysis of the role of the PI3K-Akt pathway in lipopolysaccharide-induced cytokine and tissue factor gene expression in monocytes/macrophages. *J. Immunol.* **180**, 4218–4226.
- Schabbauer, G., Luyendyk, J., Crozat, K., Jiang, Z., Mackman, N., Bahram, S., Georgel, P. (2008) TLR4/CD14-mediated PI3K activation is an essential component of interferon-dependent VSV resistance in macrophages. *Mol. Immunol.* **45**, 2790–2796.
- Schabbauer, G., Tencati, M., Pedersen, B., Pawlinski, R., Mackman, N. (2004) PI3K-Akt pathway suppresses coagulation and inflammation in endotoxemic mice. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1963–1969.
- Weichhart, T., Saemann, M. D. (2008) The PI3K/Akt/mTOR pathway in innate immune cells: emerging therapeutic applications. *Ann. Rheum. Dis.* **67** (Suppl. 3), iii70–iii74.
- Williams, D. L., Li, C., Ha, T., Ozment-Skelton, T., Kalbfleisch, J. H., Preiszner, J., Brooks, L., Breuel, K., Schweitzer, J. B. (2004) Modulation of the phosphoinositide 3-kinase pathway alters innate resistance to polymicrobial sepsis. *J. Immunol.* **172**, 449–456.
- Cantley, L. C. (2002) The phosphoinositide 3-kinase pathway. *Science* **296**, 1655–1657.
- Clausen, B. E., Burkhardt, C., Reith, W., Renkawitz, R., Forster, I. (1999) Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res.* **8**, 265–277.
- Salojin, K., Oravec, T. (2007) Regulation of innate immunity by MAPK dual-specificity phosphatases: knockout models reveal new tricks of old genes. *J. Leukoc. Biol.* **81**, 860–869.
- Hammer, M., Mages, J., Dietrich, H., Schmitz, F., Striebel, F., Murray, P. J., Wagner, H., Lang, R. (2005) Control of dual-specificity phosphatase-1 expression in activated macrophages by IL-10. *Eur. J. Immunol.* **35**, 2991–3001.
- Salojin, K. V., Owusu, I. B., Millerchip, K. A., Potter, M., Platt, K. A., Oravec, T. (2006) Essential role of MAPK phosphatase-1 in the negative control of innate immune responses. *J. Immunol.* **176**, 1899–1907.
- Suzuki, A., Yamaguchi, M. T., Ohteki, T., Sasaki, T., Kaisho, T., Kimura, Y., Yoshida, R., Wakeham, A., Higuchi, T., Fukumoto, M., Tsubata, T., Ohashi, P. S., Koyasu, S., Penninger, J. M., Nakano, T., Mak, T. W. (2001) T cell-specific loss of PTEN leads to defects in central and peripheral tolerance. *Immunity* **14**, 523–534.
- Peyssonnaud, C., Datta, V., Cramer, T., Doedens, A., Theodorakis, E. A., Gallo, R. L., Hurtado-Ziola, N., Nizet, V., Johnson, R. S. (2005) HIF-1 α expression regulates the bactericidal capacity of phagocytes. *J. Clin. Invest.* **115**, 1806–1815.
- Tanzola, M. B., Kersh, G. J. (2006) The dual specificity phosphatase transcriptome of the murine thymus. *Mol. Immunol.* **43**, 754–762.
- Knapp, S., Wieland, C. W., Florquin, S., Pantophlet, R., Dijkshoorn, L., Tshimbalanga, N., Akira, S., van der Poll, T. (2006) Differential roles of CD14 and Toll-like receptors 4 and 2 in murine *Acinetobacter pneumoniae*. *Am. J. Respir. Crit. Care Med.* **173**, 122–129.
- Renckens, R., Roelofs, J. J., Knapp, S., de Vos, A. F., Florquin, S., van der Poll, T. (2006) The acute-phase response and serum amyloid A inhibit the inflammatory response to *Acinetobacter baumannii pneumoniae*. *J. Infect. Dis.* **193**, 187–195.
- Knapp, S., Wieland, C. W., van't Veer, C., Takeuchi, O., Akira, S., Florquin, S., van der Poll, T. (2004) Toll-like receptor 2 plays a role in the early inflammatory response to murine pneumococcal pneumonia but does not contribute to antibacterial defense. *J. Immunol.* **172**, 3132–3138.
- Boone, D. L., Turer, E. E., Lee, E. G., Ahmad, R. C., Wheeler, M. T., Tsui, C., Hurley, P., Chien, M., Chai, S., Hitotsumatsu, O., McNally, E., Pickart, C., Ma, A. (2004) The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. *Nat. Immunol.* **5**, 1052–1060.
- Guha, M., Mackman, N. (2001) LPS induction of gene expression in human monocytes. *Cell. Signal.* **13**, 85–94.
- Guha, M., O'Connell, M. A., Pawlinski, R., Hollis, A., McGovern, P., Yan, S. F., Stern, D., Mackman, N. (2001) Lipopolysaccharide activation of the MEK-ERK1/2 pathway in human monocytes mediates tissue factor and tumor necrosis factor α expression by inducing Elk-1 phosphorylation and Egr-1 expression. *Blood* **98**, 1429–1439.
- Yao, J., Mackman, N., Edgington, T. S., Fan, S. T. (1997) Lipopolysaccharide induction of the tumor necrosis factor- α promoter in human monocytic cells. Regulation by Egr-1, c-Jun, and NF- κ B transcription factors. *J. Biol. Chem.* **272**, 17795–17801.
- Chi, H., Barry, S. P., Roth, R. J., Wu, J. J., Jones, E. A., Bennett, A. M., Flavell, R. A. (2006) Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase 1 (MKP-1) in innate immune responses. *Proc. Natl. Acad. Sci. USA* **103**, 2274–2279.
- Hammer, M., Mages, J., Dietrich, H., Servatius, A., Howells, N., Cato, A. C., Lang, R. (2006) Dual specificity phosphatase 1 (DUSP1) regulates a subset of LPS-induced genes and protects mice from lethal endotoxin shock. *J. Exp. Med.* **203**, 15–20.
- Lang, R., Hammer, M., Mages, J. (2006) DUSP meet immunology: dual specificity MAPK phosphatases in control of the inflammatory response. *J. Immunol.* **177**, 7497–7504.
- Brondello, J. M., Pouyssegur, J., McKenzie, F. R. (1999) Reduced MAP kinase phosphatase-1 degradation after p42/p44MAPK-dependent phosphorylation. *Science* **286**, 2514–2517.
- Laird, M. H., Rhee, S. H., Perkins, D. J., Medvedev, A. E., Piao, W., Fenton, M. J., Vogel, S. N. (2009) TLR4/MyD88/PI3K interactions regulate TLR4 signaling. *J. Leukoc. Biol.* **85**, 966–977.
- Schabbauer, G., Matt, U., Günzl, P., Warsawska, J., Furtner, T., Hainzl, E., Elbau, I., Mesteri, I., Doninger, B., Binder, B. R., Knapp, S. (2010) Myeloid PTEN promotes inflammation but impairs bactericidal activities during murine pneumococcal pneumonia. *J. Immunol.* **185**, 468–476.
- Martin, M., Rehani, K., Jope, R. S., Michalek, S. M. (2005) Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. *Nat. Immunol.* **6**, 777–784.
- Zhao, Q., Wang, X., Nelin, L. D., Yao, Y., Matta, R., Manson, M. E., Baliga, R. S., Meng, X., Smith, C. V., Bauer, J. A., Chang, H., Liu, Y. (2006) MAP kinase phosphatase 1 controls innate immune responses and suppresses endotoxin shock. *J. Exp. Med.* **203**, 131–140.
- Franklin, C. C., Kraft, A. S. (1997) Conditional expression of the mitogen-activated protein kinase (MAPK) phosphatase MKP-1 preferentially inhibits p38 MAPK and stress-activated protein kinase in U937 cells. *J. Biol. Chem.* **272**, 16917–16923.
- O'Shea, J. J., Murray, P. J. (2008) Cytokine signaling modules in inflammatory responses. *Immunity* **28**, 477–487.
- Heit, B., Robbins, S. M., Downey, C. M., Guan, Z., Colarusso, P., Miller, B. J., Jirik, F. R., Kubes, P. (2008) PTEN functions to "prioritize" chemotactic cues and prevent "distraction" in migrating neutrophils. *Nat. Immunol.* **9**, 743–752.

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PTEN • inflammation • macrophage